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Standardization and validation of a protocol of size measurements by dynamic light scattering for monodispersed stable nanomaterial characterization

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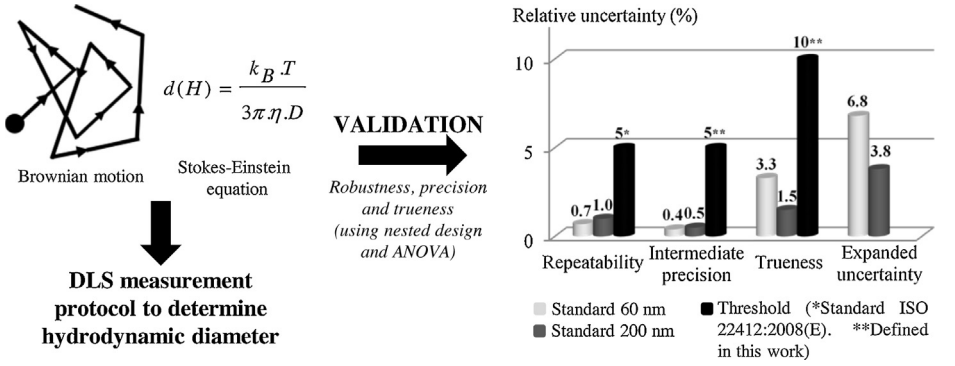
HIGHLIGHTS

- A methodology to validate protocols of size measurement by DLS was proposed.
- A validation of 2 protocols was achieved using 2 standards at 60 and 203 nm.
- Protocols were found robust, accurate and consistent with standard ISO.
- Expanded uncertainties were less than 10% for measurements performed at 25 °C.
- Protocols were suitable to determine the size of monodispersed nanomaterials.

GRAPHICAL ABSTRACT



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ABSTRACT

Among physico-chemical properties used to characterize nanomaterials, size and size distribution are essential. Several methods are suitable to evaluate these characteristics including the well-established method based on dynamic light scattering (DLS). Size measurement protocols have been rarely standardized or totally validated. However, there is a need for having standardized and validated protocols for the characterization of nanomaterials due to the pressure of authorities interested by the evaluation of risk assessments on nanomaterials that are introduced in increasing number of applications. Standardization is paramount to provide comparable values between different laboratories. This paper proposes a standardization of two protocols for evaluating the size of nanomaterials by DLS at 20 and 25 °C and a methodology to achieve their validation by investigating their robustness, precision and trueness using

Abbreviations: ANOVA, analysis of variance; AT, ambient temperature; Chol, ovine wool cholesterol; CLS, centrifugal liquid sedimentation; CRM, certified reference material; DLS, dynamic light scattering; EPC, chicken egg L-α-phosphatidylcholine; EPC/Chol, chicken egg L-α-phosphatidylcholine/ovine wool cholesterol; ERM, European Reference Materials; GUM, guide to the expression of uncertainty in measurement; IBCA, isobutylcyanoacrylate; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; IP, intermediate precision; IRMM, Institute for Reference Materials and Measurements; ISO, International Organization for Standardization; MLVs, multilamellar vesicles; NIST, National Institute of Standard and Technology; NMR, nuclear magnetic resonance; PDI, polydispersity index; PEG, poly(ethylene glycol); PLA, poly(lactide); PLA-*b*-PEG, poly(lactide)-block-poly(ethylene glycol); r, repeatability; t, trueness; TEM, transmission electron microscopy.

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Standardized protocol
Validation
Analysis of variance
Nested design

appropriate certified reference materials including standards of 60 and 203 nm whose assigned values were traceable to the International System of Units. Data were interpreted using specifically designed methods of analysis of variance ANOVA. Uncertainties of protocols proposed in the present work were 7.0 and 3.8% for dispersions at 60 and 203 nm respectively at temperature of measurement of 20 °C and 6.8 and 3.8% for dispersions at 60 and 203 nm respectively at temperature of measurement of 25 °C. These values attested that both protocols give reliable size measurements of diverse nanomaterials.

1. Introduction

Nanomaterials underwent a rapid development over the last few decades. Promises are numerous and they were already adopted for many applications in a wide range of domains including medicine. Because of their small size, they can diffuse in the body and accumulate in various organs and tissues. Although difficult to control, their small size property is used in the domain of the nanomedicine to improve drug delivery to target cells and organs with a high benefit for the treatment of severe diseases (e.g. cancer) for which no treatments based on classical formulations of drugs are yet available [1–7]. Although safety of nanomaterials developed as nanomedicines is seriously studied while developing nanomedicines, accumulation of nanomaterials in the body becomes a major health issue considering pollutants and the handling of nanomaterials in the industry. So, accurate size measurement is paramount for the reliability of obtained results for nanomaterial characterization as it is one of the nanomaterial characteristics which influences their biodistribution after entry in the body [8,9]. Thus, health agencies concerned by safety aspects of materials and chemicals have identified the size of nanomaterials as a key parameter to characterize these materials. Agencies have also identified a series of appropriate methods that they recommend to use. The method of dynamic light scattering (DLS) appeared as the preferred method. It is described in the standard ISO [10] and accessible for laboratories thanks to the existence of a series of commercial instruments. Several manufacturers have provided instruments making measurements easy to perform on dispersions of nanomaterials. In general, results give the mean size of the dispersion expressed as the mean hydrodynamic diameter (or radius) of nanomaterials assuming a spherical shape [10–12]. The method is well implanted in laboratories in both industry and academia. However, standardized protocols of the measurement of nanomaterial size were rarely described in the literature including effort on validation. One attempt of standardized protocol was proposed by the Nanomedicine Characterization Laboratory, Frederick, MD, USA [13] and only one validation of a protocol was reported in the literature [12]. There is a need to develop standardized protocols that can be applied to a wide range of nanomaterials [14] and that will be validated. Standardization of size measurement would also be needed to provide comparable values between different laboratories. It is noteworthy that results obtained using the same method should have the same measurement uncertainties if measurements were performed with a validated protocol independently of the apparatus and of the laboratory.

Accuracy of a measurement method is described using two terms "trueness" and "precision" in the standard ISO [15] and forms an integral part of validation of measurement method referring to the process to provide proof, with reference material, that a procedure is sufficiently acceptable, reliable and adequate for the different elements of its scope [9,10,13,15,16]. The validation is performed according to the procedure defined for the analysis of the sample to the evaluation of certified reference materials (CRM). Validation is necessary to prove the absence of significant bias and to estimate the expanded uncertainty i.e. the quantitative expression of the reliability of the results of a validated measurement method [16]. Based on the guidelines Q2(R1) from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH guidelines Q2(R1)), Shekunov et al. [9] summarized validation parameters applicable to nanomaterial sizing given by: (i) the precision (closeness of agreement between a series of independent test results obtained from multiple sampling of the same homogeneous sample under stipulated conditions depending only on the distribution of random errors and not related to the true value or the specified value [15–17]), (ii) the trueness (closeness of agreement between the average value obtained from a large series of test results and the value which is accepted either as a conventional true value or an accepted reference value involving systematic errors usually expressed in terms of bias [15,16]) and (iii) the working range (range of measured values whose method has proven to be reliable [16]). Usual validation parameters as robustness (measure of the capacity of a procedure to remain unaffected by small deliberate variations in measurement conditions providing indications of its reliability for normal usage [16]) has to be studied for validation of nanomaterial size measurement. Other parameters including specificity, detection limit, quantification limit and linearity are also described in the guidelines Q2(R1) [16]. However, the latter applies to evaluate performance of procedure related to quantitative methods which is not the case with DLS where they will not be adapted. It is noteworthy that the standard ISO [10] specifying nanomaterial size analysis by DLS recommend to study only repeatability and the number of samples required to perform this investigation is not specified [10]. DLS does not require calibration considering the commonly sense of this term. Nevertheless, performing periodically operational qualification and validating size measurement procedure are recommended [10,13]. The operational qualification and the validation size measurement procedure could be evaluated by performing measurements of polystyrene latex standard [10].

To our knowledge, only one paper reported the validation of an operating protocol, based on measurements performed on two reference materials i.e. colloidal silica and gold nanoparticles, applied to the characterization of the size of nanoparticles [12]. The model used in this study to make the statistical analysis of the data for the precision study did not allow to analyze in detail potential uncertainty provided by the separate factors that were considered. Indeed, this work has examined the repeatability (within day) and the intermediate precision (between day) without taking into account the different samples prepared and studied each day. Although factors being investigated were relevant, other factors that could introduce bias in the measurements such as different prepared samples were missing from this statistical analysis.

The purpose of the present work was to propose a standardized protocol to measure size of a broad range of nanomaterials by DLS and to evaluate its relevance by performing a validation at two temperatures of measurements with standards of two different sizes. To this purpose, methodology was developed for the validation of the protocols that was based on the recommendation of the